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Abstract: Many genomic assays that assess recurrence risk in early breast cancer (EBC) are prognostic, but they differ in risk group stratification, which can affect clinical utility. Prospective outcomes of >60K patients treated based on the 21-gene assay results have shown that chemotherapy may be safely omitted in EBC patents with low Recurrence Score (RS) results (RS<18). Because of its extensive validation and wide clinical use, the RS assay is a common comparator in head-to-head studies with other assays. Published / presented studies of the RS assay performed on the same tumor samples with Breast Cancer Index (BCI), EndoPredict (EP) or EP+clinical features (EPclin), MammaPrint (MMP), and/or Prosigna (ROR) assays were reviewed. Study findings were summarized descriptively. 14 studies were found that compared the RS assay with BCI (1), BCI, EPclin, and ROR (1), EP/EPclin (2), MMP (6), and ROR (4). Overall discordance in risk stratification ranged from 42% to 66% between assays. The RS assay classifies 12% of patients as high risk, compared with EP (63%), EPclin (48%), and MMP (46%) assays with dichotomous low/high risk groups, and compared with BCI (16%) and ROR (33%), assays that, like the RS assay, use three risk groups. The five most common genomic assays in clinical use for EBC risk stratify patients differently and thus are not interchangeable. Of these, the RS assay classifies the smallest proportion of patients as high risk and would therefore be expected to result in the fewest patients receiving chemotherapy.

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Summary of head-to-head comparisons of patient risk classifications by the 21-gene Recurrence Score® (RS) assay and other genomic assays for early breast cancer

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ABSTRACT

Many genomic assays that assess recurrence risk in early breast cancer (EBC) are prognostic, but they differ in risk group stratification, which can affect clinical utility. Prospective outcomes of >60K patients treated based on the 21-gene assay results have shown that chemotherapy may be safely omitted in EBC patents with low Recurrence Score (RS) results (RS<18). Because of its extensive validation and wide clinical use, the RS assay is a common comparator in head-to-head studies with other assays. Published / presented studies of the RS assay performed on the same tumor samples with Breast Cancer Index (BCI), EndoPredict (EP) or EP+clinical features (EPclin), MammaPrint (MMP), and/or Prosigna (ROR) assays were reviewed. Study findings were summarized descriptively.
14 studies were found that compared the RS assay with BCI (1), BCI, EPclin, and ROR (1), EP/EPclin (2), MMP (6), and ROR (4). Overall discordance in risk stratification ranged from 42% to 66% between assays. The RS assay classifies 12% of patients as high risk, compared with EP (63%), EPclin (48%), and MMP (46%) assays with dichotomous low/high risk groups, and compared with BCI (16%) and ROR (33%), assays that, like the RS assay, use three risk groups. The five most common genomic assays in clinical use for EBC risk stratify patients differently and thus are not interchangeable. Of these, the RS assay classifies the smallest proportion of patients as high risk and would therefore be expected to result in the fewest patients receiving chemotherapy.

INTRODUCTION

Many genomic assays available to assess recurrence risk in early breast cancer are prognostic, however, they differ in classifying patients into risk groups, resulting in potential differences in clinical utility and treatment decisions. These assays include the RS by the 21-gene Oncotype DX® Breast test, the 70-gene signature of the MammaPrint test, the 11-gene based EP/EPclin score by EndoPredict, the ROR score based on 46 of the 50 genes in the PAM50 signature by Prosigna, and the Breast Cancer Index (BCI), which combines a ratio of the HOXB13/IL17BR (H:I) genes with a 5-gene genomic grade component. These assays have been validated in distinct populations, as shown in Table 1 and Table 2. Of note, the validation cohorts of these assays differ not only in menopausal and nodal status, but also in terms of breast cancer estrogen and HER2 receptor status, and endocrine therapies received [1-12]. Differences in the validation cohort compositions are essential to consider when assays are compared with each other in terms of prognostic ability and prediction of treatment benefit.

Many of the above-mentioned multigene assays have been validated in retrospective studies such as the ABCSG 6/8 cohort for EP/EPclin and the ATAC and ABCSG8 cohorts for the ROR scores[5, 13, 14 15]. While the initial validation data for MammaPrint relied on assessments of archived tumors from heterogeneous and largely untreated patient cohorts, the prospective MINDACT trial has yielded higher quality prognostic validation evidence from randomized patient cohorts [11, 16, 17]. The identification of patients with high clinical risk and low genomic score with at least a 92.5% chance of being free of distant metastasis without chemotherapy at 5 years was demonstrated using the Mammaprint assay in the MINDACT trial[17]. Prospective outcomes in >60,000 patients (including both clinical and epidemiological data) treated based on 21-gene assay results have shown that patients within the low RS group (RS<18) have excellent outcomes without chemotherapy [18-24]. The prognostic power of the RS was validated in an endocrine treated NSABP B14 cohort, in the Kaiser Permanante, JBCRG, SWOG 8814 and in the transATAC sudies [8], [25].[3, 26, 27 1]. The predictive power of RS for the prediction of chemotherapy benefit was initially validated in the NSABP B20 study in endocrine only vs. chemoendocrine treated cohorts. [9]. Additionally, the recently published prospective TAILORx study demonstrates the ability to safely avoid adjuvant chemotherapy

in a larger proportion of patients with node negative disease, those with RS results from 0-25 [28]. The power of chemotherapy benefit using the Mammaprint test was tested in the MINDACT trial 2016[17] . Prediction of prognosis in chemotherapy treated patients was validated for the RS in the NASBP B28, PCAS-01, for Prosigna in the DBCG77B trial. [29] and for Endopredict in the GEICAM/9906 trials [28 30 31]. The prognostic power of Mammaprint in untreated patients was validated in the TRANSBIG study[16] . Because of its extensive validation and wide clinical use, the RS assay is a common comparator in head-to-head studies with other assays[4, 32-44].

In this retrospective systematic literature review, we addressed the question of discordance in genomic risk group classifications between the five commercially available tests. We reviewed and summarized results from 14 available studies in the literature, which compared distinct genomic scores and corresponding risk group classifications with each other when two or more assays were performed on the same tumor sample, and we used the RS as a basis for the comparison.

MATERIALS AND METHODS

Fourteen published and presented studies comparing the RS assay performed on tumor samples from the same patient cohorts with Breast Cancer Index (BCI), EndoPredict (EP) or EP+clinical features (EPclin), MammaPrint (MMP), and/or Prosigna (ROR) assays were identified and reviewed. Only studies with available data on discordance between different risk group classifications of patients between assays were included. Study findings were summarized descriptively. Distribution of risk group categories were extracted, and discordance between assays was determined. Overall discordance was defined as any difference in risk group classification, minor discordance as the difference of one risk category (low to intermediate, intermediate to low, high to intermediate, or intermediate to high), and major discordance as the difference of more than one risk category (low to high or high to low) between the RS assay and other. Additionally, an overview on five commercially available genomic tests (Mammaprint, Oncotype DX, Breast Cancer Index, Prosigna, Endopredict) was conducted with the focus restricted to validation sets and resulting classification scores (Table 1).

RESULTS

Characteristics in validation cohorts of existing multigene assays

The validation cohorts for the five multigene tests show distinct differences in risk group classifications that potentially affect prognostic utility of the assays. Pre- and postmenopausal status was included for all assays, however cut-off for definition of menopausal status (50 years vs 60 years), nodal status, inclusion of ER and/or HER2 status as well as tumor size differs in the different assays [1-8, 10-12, 45]. As to nodal status, except for the BCI assay, node negative and node positive patients were both included in all cohorts. It is of note that the definition of nodal positivity differs; pN2 patients were included in the EP/EPclin score validation, but not in the validation cohorts for the other assays. Hormone receptor status also shows distinct differences as patients of any ER status were included in two tests (Mammaprint and Prosigna) whilst two other tests (Oncotype and Endopredict) included only ER positive patients for the validation design [1-8, 10-12, 45]. The RS was initially validated in pre- and post-menopausal, node-negative ER-positive patients treated with 5-years of hormonal therapy; however, subsequent validation evidence and prospective outcomes data have been generated in both node-negative and node-positive ER-positive, HER2-negative early breast cancer patients treated with adjuvant hormonal therapy (Detailed description of the multigene tests are shown in **Table 1 and Table 2).**

A short summary of development of multigene tests follows:

Mammaprint

This 70 gene multigene test was first validated in 2002 on a virtually untreated cohort of young patients (less than 55 years-old with pT1/pT2 tumors, nodal negative/positive status, most not receiving adjuvant systemic therapy regardless of nodal status) [46]. In the consecutive validation study, most node positive and some of the node negative patients received adjuvant systemic therapy [46]. Genes included in Mammaprint are associated with proliferation, invasion and angiogenesis[47]. The test is performed centrally, conducted in Amsterdam, Netherlands. Mammaprint provides low and high risk genomic scores without including clinical parameters. High clinical risk coupled with low genomic profile identifies a heterogeneous group of patients with a risk of distant metastases at 5 years that likely does not exceed 7.5% [2, 7, 46]. The difference between clinical and genomic risk groups (as low

genomic /high clinical risk versus high genomic/low clinical risk) was found not to differ significantly and showed no relevant effect in predicting chemotherapy benefit [11, 16, 17].

Oncotype DX

This 21 gene test was initially validated on the NSAPB-B14 and NSAPB-B20 prospective clinical trials including nodal negative and hormone receptor (ER) positive early breast cancer (pT1-2) patients receiving endocrine (tamoxifen) therapy (NSABP-B14) and endocrine (tamoxifen) or chemoendocrine therapy (NSABP-B20) [3, 8, 9]. This is a centralized test as well, currently performed at Genomic Health (Redwood City, CA, USA). Involved genes are related to ER, HER2, proliferation and invasion/metastases. Oncotype DX provides continuous RS results between 0-100, providing information on distant recurrence at 10 years in all ER positive HER2 negative patients independently of tumor size or of nodal status [1]. Predictive power of the RS test was repeatedly confirmed in clinical trials[1, 9, 48]. High RS results (RS >25) are predictive for large chemotherapy benefit[9]. On the contrary, the prospective TAILORx study recently reported 9-year outcomes showing no benefit from chemotherapy for node negative, ER positive HER2 negative patients with RS 11-25, and confirming excellent outcomes for those with RS 0-25 treated with hormone therapy alone. [1, 9, 48]

Breast Cancer Index (BCI)

This test of 5+2 genes was initially validated in 2008 and represents the combination of the molecular grade index (MGI) and the HOXB13:IL17BR ratio (H:I) [49].

Genes in BCI are prognostic, associated with clinical outcome and the assay is performed centrally at Biotheranostic, San Diego, USA[49]. BCI reports low and high risk scores (between 1-10) without the inclusion of clinical parameters. Patients of all age, with ER+/ HER2 negative and nodal negative breast cancer undergoing adjuvant hormonal therapy are eligible for testing. High risk BCI can prognosticate distant recurrence at 5 years respectively after 10 years [10, 12, 49-51 52]. No prospective data on BCI are available for predictive power of treatment benefit.

Prosigna

This 50 (50+8 reference genes) gene test was initially validated in 2009 in a postmenopausal patient subset independently of nodal status including all intrinsic subtypes [53]. Genes in the Prosigna test are related to various molecular pathways and to tumor proliferation [54]. The test is decentralized and can be performed by local pathology laboratories. The Prosigna test provides not only continuous ROR risk scores (scale 0-100) but also the intrinsic subtype (Luminal A or B, HER2, Basal-like). The ROR score is calculated using coefficients from a Cox model that includes the Spearman correlation of a 46-gene subset of the 50 genes to each intrinsic subtype centroid, a proliferation score, and gross tumor size (< 2 cm or > 2 cm). Risk categories (low, intermediate, high) are derived from the ROR score and the nodal category (0 or 1–3 positive nodes). Additionally, the test has been approved by the FDA for use in postmenopausal patients. The prognostic value of ROR scores has been demonstrated in the transATAC and ABCSG08 studies, showing that the low ROR or Luminal A intrinsic subtype is associated with a very low 10 years distant recurrence[4, 6, 14, 53]. More recently, the Prosigna ROR score and intrinsic subtypes were shown to be prognostic in high-risk premenopausal patients with early breast cancer, and intrinsic subtype was predictive for adjuvant C/CMF treatment in this study [55] . No prospective data on Prosigna are available for predictive power for treatment benefit however.

Endopredict

This 11 +1 gene test was validated in 2011 in a ABCSG06 and ABCSG08 cohorts of ER positive postmenopausal patients independent of nodal status including pN0/pN1-pN2 patients as well [5]. Genes in the Endopredict test are ER and proliferation related genes. The test is de-centralized and can be performed in local pathology laboratories. The Endopredict test provides the EP score (0-15) and the EPclin score: the latter is a combination of EP Score + pT + pN. [5]. The prognostic value of the Endopredict test is currently available as the EPclin score, whilst EP score is not reported separately in the commercially available tests. EPclin score can prognosticate 10 years distant recurrence, which is 4% at low EPclin and and 28% at high EPclin (group average in a particular study population). [5, 13, 31]. No data on predictive power for treatment benefit are available for Endopredict.

Characteristics of the 14 studies enrolled in the review

Fourteen studies ranging in size from 34 to 1007 patients were included in the current review, summarizing 5514 patients. Data on discordant results were available in 10 of 14 studies. RS results were compared with BCI in one study, with MMP in four studies, with ROR in four studies, and with EP/EPclin in one study [4, 32, 33, 35, 37-39, 41, 42], [44]. One further study, the 2016 TransATAC study, compared RS with BCI, ROR, and EPclin [39]. As to nodal status, three of 14 studies included node-negative patients only (RS vs BCI in TransATAC 2016, RS vs ROR by Alvarado et al 2015, as well RS vs MMP by Maroun et al. 2015), whilst 11 of 14 studies included node-positive patients as well [32, 38, 39].
A list of the studies is shown in **Table 3**.

Discordance between the assays

Overall discordance in risk categorization between the RS assay and other genomic assays ranged from 42% to 66%. The most frequent comparisons were conducted between the RS assay and MMP (n=4) and the RS assay and ROR (n=3). In comparisons of the RS assay vs MMP, minor discordances ranged from 26% to 38% of cases, while major discordances ranged from 19% to 25% of cases. In comparing the RS assay vs ROR, minor discordances ranged from 37% to 45% of cases, while major discordances ranged from 3% to 20% of cases. In the only available study comparing the RS assay vs. EP/EPclin, minor discordance was detected in 29% of cases, while major discordance ranged from 18% to 21% of cases, and overall discordance was seen in 47%-50%. Comparing the RS assay vs BCI, minor discrepancies occurred in 37% and major discordance in 5% of the cases. A summary of the discrepant classification using different multigene assays is shown in **Table 3.**

Risk group distribution by studies

Among all studies, high-risk genomic score was classified in a range from 11.5% to 63% of patients. Of these genomic assays, the RS assay classifies the smallest proportion of patients as high risk (11.5%), whilst EPclin classifies the highest percentage of patients as high risk (63% for EP and 48.4% for EPclin). Even when compared with ROR and BCI, both of which, like the RS assay, classify patients into three risk categories, the RS test results in the lowest percentage of high-risk patients. For BCI, the intermediate group is as defined by Sestak et al[15]. A summary of individual risk classification is shown in **Figure 1** and **Figure 2.**

Discussion

In this review, we compared results of studies that reported comparisons of risk classifications of the 21-gene assay with one of four additional commercially available genomic tests used for treatment decision-making in early breast cancer. This review demonstrates that RS results classify the lowest proportion of patients as high-risk at 11.5% whilst EPclin classifies the highest proportion of patients as high-risk on the same tumor tissue at 63%. However, it is not clear if these discrepancies may result in over-treatment or under-treatment of patients.

Differences in risk group classifications may arise from clinical and biologic differences in original study populations from which each assay was developed and validated as well as from different approaches to defining pre-specified cut-points across scores, and differences in the genes measured.

The validation cohort for the EPclin assays was composed solely of postmenopausal ER+ / HER2- study patients[5, 6, 53]. The validation cohort for the Prosigna assay also included postmenopausal patients independently from intrinsic subtypes[5, 6, 53],[14 6]. As such, any "high risk" groups delineated by these tests may not be at the same level of risk when broader ER+ populations that include premenopausal patients or more obvious chemotherapy candidates are considered. Furthermore, different approaches to pre-specifying cut-points across scores also influence risk group classifications: assays defining dichotomous "high" or "low" risk groups, while seemingly convenient, may lose some accuracy in classifying patients whose recurrence risks are near those of the cut-point.

Though all assays are based on gene expression algorithms producing composite scores that correlate with risks of recurrence, the assays may perform differently based on differences in genes and biologic pathways being measured as well as analytic factors that affect precision and accuracy. The latter factors include performance characteristics of genomic assessment platforms, quality control methods, and even differences in pre-analytic factors inherent in the processing of tissues for each assay [3, 33, 44]. [54].

The OPTIMA study conducted four prognostic tests on the same tumor blocks, including three genomic tests: RS by Oncotype DX, ROR by Prosigna and MammaPrint as well as

IHC4 [33]. In this comparative study, each test classified patients differently, with relatively low overall agreements between any two tests based on Kappa values varying between 0.33-0.60 [33]. The highest percentage of low-risk genomic score was achieved by RS (81%), followed by IHC4 (72%), ROR (65%), and MammaPrint (61.4%) [33]. In the transATAC study, a similar overall discordance rate of 42% was described in a node-negative hormone receptor-positive patient population when BCI was compared with RS, resulting in reclassification (p=0.003), especially in the low-intermediate prognostic groups [39]. A direct comparison of RS with EPclin scores by Buus et al. suggested superiority of EPclin in terms of prognostic information over RS; however node-positive (pN2) patients were included in this study as well [44]. Since the EPclin result relies on an algorithm that heavily weights nodal status, it may be expected that the prognostic differences for high- and low-risk groups might be greater when nodal status is incorporated [44]. Another study by Dowsett et al reported superiority of ROR in prognostic information to RS; however, more patients were classified as high risk by ROR than by RS [4]. The inclusion of pN2 samples in validation cohorts might drive up the high-risk numbers.

Patients identified as “high-risk” by any multigene assay are most likely to receive chemotherapy, with clear implications for toxicity, quality of life, treatment costs, and the likelihood of being over-treated. While each assay identifies patients at higher risks for recurrence, only the RS test has shown that their high-risk group represents patients for whom DFS and distant recurrence outcomes with chemotherapy treatment are superior to those achieved with hormone therapy alone. [1, 9]

An emerging number of papers have addressed the impact of multigene tests on adjuvant chemotherapy decisions by physicians. A recently published paper comparing MammaPrint and EndoPredict risk stratification showed a poor correlation between the two tests and noted that following EndoPredict results in the study, a change in therapy decisions in favor of adding chemotherapy to hormone therapy would have occurred in 38% of the patients [56]. Another recent study by the SAKK 26/10 analyzed the decision impact of Oncotype DX testing and reported a change in therapy decision against chemotherapy and in favor of hormone therapy alone in 44% of the patients [57]. A similar recent UK study reported a change against chemotherapy in favor of endocrine therapy alone in 69.2% of patients after

the Oncotype DX results were discussed in an interdisciplinary meeting [58]. Interestingly, the West German Breast Cancer Study Group found that using the Prosigna assay resulted in 29.3% discrepant intrinsic subtyping compared to routine IHC, with a change in therapy decisions in 18.2% of the patients, two-thirds (22 of 36 patients) switched from no chemotherapy to chemotherapy [59].

Conclusion

The five most common genomic assays in clinical use for early breast cancer each risk-stratify patients differently, thereby carrying implications for the potential use of adjuvant chemotherapy. The proportion of patients classified as high risk differs in these tests, however it remains unclear whether this may lead to under- or overtreatment. Even when the risk group distribution appears similar between assays, there is considerable discordance in patient classification into these groups. As such, these assays should not be used interchangeably. Ultimately, the clinical utility of the individual genomic scores is best assessed in adequately powered prospective clinical trials that address a specific practical management issue.

Acknowledgement /conflict of interest

ZV received consultancy and speaking fees from Roche, Astra Zeneca and Genomic Health. PS purchased equipment from Nanostring and received consultancy fees from Genomic Health. AS served as consultant and speaker for Genomic Health.

Legend of Figures

Figure 1. Pooled risk group distribution.
RS groups defined as low (RS<18), intermediate (RS 18-30), and high (RS ≥ 31)

Figure 2. Risk group distribution, by each study in the current comparison.
[a] For BCI, the intermediate group is as defined in Sestak 2016 [39]. [b] The Tsai 2018 study sought to reclassify by MMP patients who were first classified as intermediate by the RS assay [43]. RS groups defined as low (RS<18), intermediate (RS 18-30), and high (RS ≥ 31).

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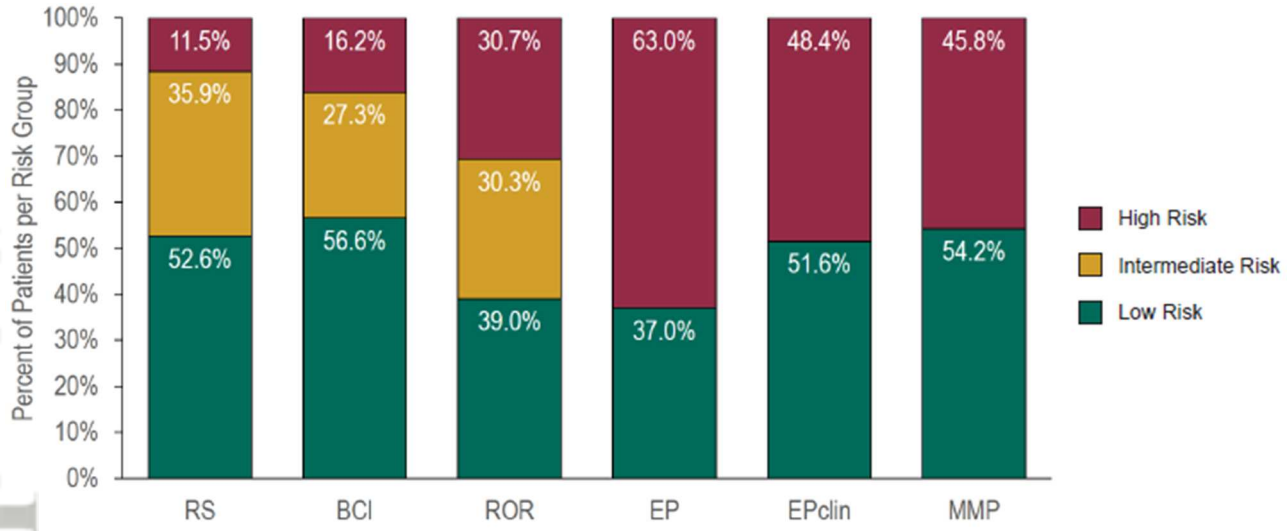

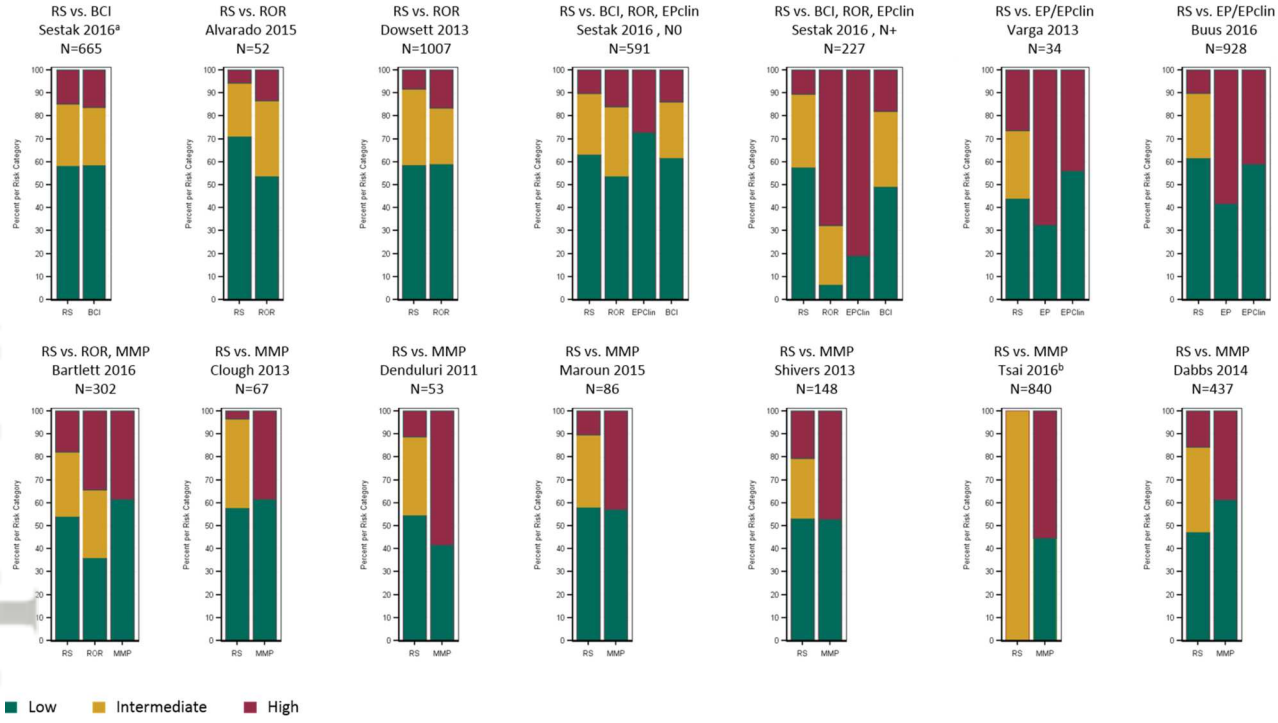

Table 1. Characteristics of the five multigene tests in terms of validation and methodology, genes tested, evidence for prognosis / prediction and clinical indication.

Multigene Test	**Mammaprint**	**Oncotype Dx**	**Breast Cancer Index**	**Prosigna**	**Endopredict**
Year validated	2002	2004	2008	2009	2011
Validation paper first published	van't Veer et al. Nature 2002 (Ref. 46)	Paik S et al. N Engl J Med 2004 (Ref. 8)	Ma XJ et al. Clin Cancer Res 2008 (Ref. 49)	Parker et al J Clin Onc 2009 (Ref. 53)	Filipits et al Clin Cancer Research 2011 (Ref. 5)
Validation set	n= 97 cases (LN -, < 5cm, <55 years)	N=668 cases (LN -, ER +, adjuvant ET)	n=410 and n=323 (ER +, HER2 -, adjuvant ET)	N=189 cases (LN +/-, all subtypes) Postmenopausal status	N=964 cases (LN +/-, >55 years, adjuvant ET, HER2 -) Postmenopausal status
Nr. of genes	70 genes	21 genes (16+5)	5 + 2 genes	58 genes (50+8)	11 (8+3) +1 genes
Tissue type used	frozen tumor tissue	FFPE tissue	FFPE tissue	FFPE tissue	FFPE tissue
Genes tested (# of genes)	12 groups (Ref. 47): Apoptosis (2) Antigrowth signaling (1) Growth factors (6) Proliferation and oncogenic transformation (12) Cell cycle (15) Adhesion and remodeling (5) Motility (3)	6 groups (Ref. 8): ER-related (4) Proliferation (5) HER2-related (2) Invasion (2) Others (3) Reference (5)	3 groups (Ref. 49): H:I index (2 genes) Molecular grade (5) Reference (4)	13 groups (Ref. 54): Proliferation (18) Cytoskeletal function (4) Aptotosis (2) RTK- and proliferation-signalling (3) ECM & adhesion (5) HER2 signaling (2) Cell division (3) Hormone signaling (3) WNT-pathway (2)	3 groups (Ref. 5): Proliferation (3) Hormone-receptor related (5) Reference (4)

	Altered metabolism (7) Angiogenesis (6) Unknown function (9) Miscellaneous (7)* *3 genes were assigned to more than one group			Metabolism (4) Ubiquitin ligase (1) Miscellaneous (3) Reference (5)	
Methodology	DNA microarrays	qRT-PCR	qRT-PCR	Nanostring nCounter	qRT-PCR
Test availibility	central	central	central	de-central	de-central
Genomic scores	Genomic risk score	Recurrence Score (RS 0-100)	Risk score (0-10)	ROR Score (0-100) Intrinsic subtype	EP Score (0-15) EPClin Score (1 - 6,9)
Risk category	Low High	Low Intermediate High	Low High	Low Intermediate High	Low High
Clinical parameters	No clinical parameters taken into account of risk scores	Interpretation of RS using pN -/+ RSPC= RS + age+ pT + Grade (N0)	No clinical parameters taken into account in risk scores	ROR = PAM50 + pT (+/- 2cm) + pN (-/+)	EPclin = EP score + pT + pN
Indication for testing	Invasive breast cancer pT1-2 pN0/pN1 age < 55 years	Invasive breast cancer pT1-2 ER+ / HER2- pN0/pN1	Invasive breast cancer pT1-2-3 ER+ / HER2- pN0 adjuvant ET	Invasive breast cancer pT1-2 / pN0 or pT2 /pN1 ER+ adjuvant ET postmenopausal status	Invasive breast cancer pT1-2 ER+ / HER2- pN0/pN1 postmenopausal status

Validated for prognosis	Yes van't Veer 2002 (Ref.46) van de Vijver 2002 (Ref.11) Bueno-de-Mesquita 2007 (Ref.2) Mook 2009 (Ref.7)	Yes Dowsett 2010 (Ref.3) Albain 2010 (Ref.1) Paik 2004 (Ref.8)	Yes Ma 2008 (Ref.49) Zhang 2013 (Ref.12) Sgroi 2013 (Ref.10) Sgroi 2016 (Ref.50)	Yes Parker 2009 (Ref.53) Dowsett 2013 (Ref.4) Gnant 2014 (Ref.6) Gnant 2015 (Ref.14)	Yes Filipits 2011 (Ref.5) Martin 2014 (Ref.31)
Results of prognostic validation	High risk genomic profile is prognostic for distant metastases in 5 years in -pN0, -age <55 years, -no chemotherapy subgroup	Low risk RS (<18) and high risk RS (at least 31) are prognostic for distant recurrences at 10 years in patients: - all age, - all tumor size, - pN0/pN1. -ER+/Her2-	High risk BCI is prognostic for 5 resp. > 10 years distant recurrence in patients: -all age -pN0 -ER+/Her2-	Low ROR with Luminal A intrinsic subgroup is prognostic for low 10 years distant recurrence in patients: -pN0/pN1 -pT2 -ER+/Her2- -postmenopausal status	EPclin low and EPclin high risk are prognostic for 10 years distant recurrence (4% vs.28%) in patients: --pN0/pN1 - pT1/pT2 -ER+/Her2- -postmenopausal status
Validation trials for prediction	Yes Cardoso 2016 (Mindact) (Ref.17).	Yes Paik 2006 (Ref.9) Albain 2010 (Ref.1) Sparano 2018 (TailorX) (Ref.48)	No	No	No
Results of prediction validation	High clinical risk coupled with low genomic profile identifies a heterogeneous group of patients with a risk of distant metastases at 5 years that likely does not exceed 7.5%. Chemotherapy benefit in this group is 1.5%.	High risk RS (at least 31) and intermediate risk (>25) are predictive for additional chemotherapy benefit. Low risk RS (<18) is predictive for minimal if any benefit, in patients: ->55 years (high and low risk)	-	-	-

		>50 years (intermiediate risk), -of all tumor size, -in pN0 -ER+/Her2-			

Abbreviations : ET (endocrine therapy), ER (estrogene), FFPE (formalin fixed paraffin embedded), LN (nodal status)

Table 2. Summary of major clinical validation studies on multigene tests

		Trial	author / published in	Overall risk	HR
Prognostication in endocrine treated patients					
Oncotype DX	n=668, ER+, pN0	NSABP B14	Paik / NEJM 2004 (Ref.8)	DRFS 10y- 85%	2.81 RS low 6.8% RS int 14.3% RS high 30.5%
Oncotype DX	220 cases, 570 controls, ER+, pN0	Kaiser Permanente	Habel / Breast Cancer Research 2006 (Ref.25)	OS 10y- 92.7%	Relative risk RR=2.4 RS low 2.8% RS int 10.7% RS high 15.5%
Oncotype DX	ER+ pN0 = 200 ,pN+=80	JBCRG	Toi / Cancer 2010 (Ref.27)	DRFS 10y- 91.5%	6.03 RS low 3.3% RS int 0 % RS high 24.8%
Oncotype DX	ER+ pN0=872, pN+=306	transATAC	Dowsett / JCO 2010 (Ref.3)	pN0 DRFS 9y- 91.% pN+ DRFS 9y 76%	pN0: 5.25 pN+: 3.47
Oncotype DX	ER+ n=148 (HR therapy arm)	SWOG 8814	Albain / Lancet Oncol 2010 (Ref.1)	pN+ OS: 68-2%	4.42
Prosigna	ER+ pN0=739, pN+=268	transATAC	Sestak / JCO 2015 (Ref.15)	pN0 DRFS 10y- 89.3.% pN+ DRFS 10y 69.8%	pN0: 4.78 pN+ (1-3): 1.98
Prosigna	ER+ pN0= 1047, pN+	ABCSG08	Gnant / Ann Oncol 2014 (Ref.6)	pN0 DRFS 10y- 92.% pN+ DRFS 10y 84%	2.85
Prosigna	ER+ / HER2- pN0=1,163, pN+=1395	DBCG 77B	Lænkholm / JCO 2018 (Ref.29)	pN0 ROR low / int. / high: 95% / 92.7% / 82.2% pN+ ROR low / int. / high: 96.5% / 88.5% / 79.1% pN0/+ Luminal A / B:	0.56 (low vs. intermediate) 1.79 (high vs. intermediate) 1.92 (Luminal B vs.

				92.4% / 81.6%	A)
Endopredict	ER+ n= 378, pN0 (60%)	ABCSG06	Filipits / Cin Cancer Res 2011 (Ref.5)	DRFS 10 y -88%	1.19 EPclin low risk 4% EPclin high risk 28%
Endopredict	ER+ n=1324, pN0 (70.6%) pN+: 29% (pN1 26.4%, pN2 2.8%)	ABCSG08	Fitzal / Br J Cancer 2015 (Ref.13)	DRFS 10 y- 93%	1.16-1.48 (1.26) EP High risk 91% EP Low risk 97.5%
Prediction of chemotherapy benefit					
Oncotype DX	n=651 randomized-retrospective	NSABP-20	Paik / JCO 2006 (Ref.9)	10y DRFS: 92.2% Chemo+ TAM 87.8% Tam alone	Chemotherapy benefit: Low RS:1.31 High RS: 0.26
Oncotype DX	n=367 randomized-retrospective	SWOG 8814	Albain / Lancet Oncol 2010 (Ref.1)	10y DRFS: 64.8% chemo + TAM 55.4% Tam alone	Chemotherapy benefit: Low RS:1.02 High RS: 0.52
Oncotype DX	n=19719 ER+/HER2- pN0 RS 11-25 randomized-prospective	TailorX	Sparano / NEJM 2018 (Ref.48)	9y DFS 83.3% endocrine therapy 84.3% chemoendocrine therapy	Chemotherapy benefit at RS:16-25 Age <50y
Prosigna	n=460 premenopausal, high risk (either N+, >5cm or	DBCG 77B	Jensen / BCR 2018 (Ref.55)	10y OS 59.7% with C 62.2% with CMF	Chemotherapy benefit (C/CMF): Luminal B: 0.61

	infiltrating fascia) randomized-retrospective			41.1% with levamisole 46.0% control arm	Basal-like: 0.44
Mammaprint	ER+/- (88.4% vs.11.6%) HER2 +/- (93% vs. 9.5%) n=6693 pN0 (79%) pN+: 21% (pN1 20.9%, pN2 0.1%) randomized-prospective	Mindact	Cardoso / NEJM 2016 (Ref.17)	Chemotherapy benefit: Low genomic/ High clinical: 1.5%	High clinical / low genomic 0.63 (DFS), 0.64 (OS) Low clinical / High genomic 0.74 (DFS) 0.72 (OS)
Prognostic in chemotherapy treated patients					
Oncotype DX	n=1019	NSABP B28	Mamounas / J Nat Can Inst 2017 (Ref.28)	10y DFS: 59%	RS: 2.59 Nodal status: 1.91
Oncotype DX	n=530	PACS01	Penault-Llorca / BMC Cancer 2018 (Ref.30)	5-y DFS 73.2% with FEC 78.4% with FEC-D	RS: 2.66 Nodal status: 2.65
Endopredict	n=556	GEICAM/9906	Martin / BREA 2014 (Ref.31)	10 y MFS; 78.5%	EP: 1.126 Nodal status: 1.4-3.6
Untreated patients					
Mammaprint	n=307	TRANSBIG	Buyse / J nat Cancer Inst 2006 (Ref.16)	DFS: 55%	2.32 (DFS) 2.79 (OS)

Overall Risk reflects the outcomes for patients receiving ET (endocrine therapy) alone, who had "favorable" or "low" genomic scores.

Abbreviations: ER: estrogene receptors, DRFS: Disease and recurrence free survival, DFS: Disease free survival, OS: overall survival. MFS: metastasis free survival.HR: hazard ratio (unit changes).

Table 3. Discordance between genomic assays

		Discordance[a] Between the RS Assay and Other Assays											
	n=	BCI			ROR			EP / EPclin			MMP		
		Minor (L ↔ I or I ↔ H)	Major (L ↔ H)	Overall	Minor (L ↔ I or I ↔ H)	Major (L ↔ H)	Overall	Minor (L ↔ I or I ↔ H)	Major (L ↔ H)	Overall	Minor (L ↔ I or I ↔ H)	Major (L ↔ H)	Overall
TransATAC (Sestak 2016) (Ref. 39)	665	37%	5%	42%									
OPTIMA (Bartlett 2016) (Ref. 33)	302				40%	10%	50%						
Marin General Hospital (Alvarado 2015) (Ref. 32)	52				37%	10%	46%						
TransATAC (Dowsett 2013) (Ref. 4)	1007				41%	3%	43%						
Heidelberg cohort (Sinn 2017) (Ref. 42)	47				45%	20%	66%						
Swiss Study (Varga 2013) (Ref. 44)	34							29% / 29%	18% / 21%	47% / 50%			
French Study (Svedman 2013) (Ref. 35)	67										38%	19%	57%
US Oncology/UCSF Study (Denduluri 2011) (Ref. 37)	53										34%	25%	58%
McGill US Study (Maroun 2015) (Ref. 38)	86										31%	22%	53%

Florida Study (Shivers 2013) (Ref. 41)	148										26%	19%	44%

[a] Overall discordance=any difference in risk classification between the RS assay and other; minor discordance=difference of one risk category (low ↔ intermediate or intermediate ↔ high); major discordance=difference of more than one risk category (low ↔ high).